



Short communication

Initial occurrence of *Taylorella asinigenitalis* and its detection in nurse mares, a stallion and donkeys in KentuckyB.J. Meade^{a,e,*}, P.J. Timoney^a, J.M. Donahue^b, A.J. Branscum^c, R. Ford^d, R. Rowe^e^a Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546, USA^b Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY 40546, USA^c Department of Biostatistics, Statistics, and Epidemiology, University of Kentucky, Lexington, KY 40536, USA^d Office of the State Veterinarian, Kentucky Department of Agriculture, Frankfort, KY 40601, USA^e United States Department of Agriculture, Animal Plant Health Inspection Services, Veterinary Services, Frankfort, KY 40602, USA

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ABSTRACT

In 1998, a newly identified bacterium *Taylorella asinigenitalis* was isolated from the external genitalia and reproductive tracts of nurse mares, a stallion and donkey jacks in Kentucky. An extensive regulatory effort was implemented to contain the outbreak including the tracing and testing of 232 horses and donkeys on 58 premises. *T. asinigenitalis* was isolated from the reproductive tract of 10 adult equids, including two donkey jacks, one Paint Quarter-horse stallion and seven draft-type breeding mares. None of the infected horses had clinical signs of reproductive tract disease. The odds of being culture positive were 20 times greater for a mare bred to a donkey than for a mare bred to a stallion. Approximately 18% of mares bred to either a carrier stallion or donkey jack were confirmed culture positive. Seventy-one percent of infected mares required more than one course of treatment to clear the organism from their reproductive tracts and one mare harbored the organism for more than 300 days.

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1. Introduction

1.1. Background

Contagious equine metritis (CEM) is a contagious venereal disease of equids caused by the bacterium *Taylorella equigenitalis* (Platt and Taylor, 1982; Timoney and Powell, 1988; Timoney, 1996). Infection in the mare may be symptomatic or subclinical. When present, clinical disease is characterized by an endometritis, cervicitis, vaginitis of variable severity, mucopurulent vaginal discharge and temporary infertility (Timoney and Powell, 1988; Timoney, 1996). In contrast, stallions are asymptomatic carriers har-

boring the organism on their external genitalia; they are believed to serve as the primary reservoir for this bacterium (Timoney and Powell, 1988).

T. equigenitalis was first reported in the United States (US) in 1978 following the importation of two carrier Thoroughbred stallions into Kentucky (Bryans and Hendricks, 1979). Following the implementation of regulatory control measures, the disease was successfully eradicated from the US equine population but small-scale outbreaks subsequently occurred in Missouri in 1979 (Fales et al., 1979) and in Kentucky in 1982 (Donahue and Smith, 1982).

In late October 1997 and early January 1998, an organism closely resembling *T. equigenitalis* was isolated from the semen of a mammoth jack in California and the reproductive tract of several nurse mares, a stallion and two donkey jacks in Kentucky, respectively (Jang et al., 2001). Both isolates were streptomycin sensitive. The California and Kentucky isolates represented separate strains of a new species of *Taylorella*, designated *T. asinigenitalis*,

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neither of which was linked with the occurrence of clinical disease (Jang et al., 2001). Experimentally however, two mares exposed to one of the Kentucky isolates (UK-1) of *T. asinigenitalis* by the intra-uterine route developed a detectable vaginal and cervical discharge which was less intense than that observed in mares challenged with strains of *T. equigenitalis* (Katz et al., 2000).

A case report from Sweden documented the first isolation of *T. asinigenitalis* from a stallion in Europe (Båverud et al., 2006). The authors proposed that the stallion acquired the organism from a donkey jack while these animals shared a paddock in Belgium. More recently, *T. asinigenitalis* was detected in two donkey jacks in Italy (Franco et al., 2009).

What limited studies have been carried out on *T. asinigenitalis* to date has largely focused on diagnostic methods and aspects of the molecular biology of the bacterium. No studies have been reported describing patterns of infection or the transmission dynamics of *T. asinigenitalis* in exposed populations. The purpose of this report is to provide the investigative findings of the first recorded occurrence of *T. asinigenitalis* in domestic equids in the US, to quantify the potential risk of spread of *T. asinigenitalis* among various equine subpopulations and, where relevant, compare it to *T. equigenitalis*.

1.2. Investigative summary

In early January 1998, regulatory officials in Kentucky were notified by the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) that two mares on the same premises had titers of 1:16 and 1:8 in the complement fixation test (CF) to *T. equigenitalis*. The individuals were part of a group of five mares, mostly nurse mares. The two mares were being tested prior to qualifying them as test mares for post-entry breeding to an imported stallion. While the two mares had no previous exposure to any imported animal, they had been bred to a leased grey donkey jack 19–24 days prior to being sampled for CEM. Subsequently, swabs were collected from the external genitalia of the grey jack and a blood sample was obtained for CF testing. The swabs were culture positive for a CEM-like organism.

In light of the breeding history of the grey jack, the epidemiologic investigation was expanded to include the premises of origin of the grey jack. The principal business of the owner of the grey jack was to provide nurse mares, test mares and teasers to breeding farms within and outside Kentucky. In excess of 100 animals were leased yearly, mainly to Thoroughbred and Standardbred farms in the central Kentucky area. An estimated 40% of the leased mares return to the farm pregnant. Herd additions were purchased at sales and maintained in isolation for a variable period of time, usually months, before being leased.

The grey jack was treated in accordance with standard protocol for testing CEM carrier stallions, as prescribed in Title 9, Code of Federal Regulations (9 CFR), Part 93, after which it was returned to its farm of origin where the jack remained under quarantine for further culturing and test breeding.

Under the authority of the Kentucky State Veterinarian in cooperation with the United States Department of Agriculture, Animal Plant Health Inspection Service, Veterinary Services (USDA-APHIS-VS) and the University of Kentucky, Department of Veterinary Science, an investigation was undertaken of various premises owned or managed by the nurse mare provider, including any premises known to have received leased animals from this provider within the previous year.

2. Materials and methods

2.1. Regulatory protocol

Blood samples were initially collected for serological (CF) testing for antibodies to *T. equigenitalis* from equids on all premises with a direct epidemiological link to the culture-positive grey jack. This measure was considered necessary to obtain a census, identify any permanent markings for each animal, and to document possible exposure to *T. asinigenitalis*. For regulatory purposes, animals identified as having an increased risk of exposure were classified as either high or low-risk based on their association with other known *T. asinigenitalis* culture-positive animals. High-risk animals were those that had been exposed to a known positive animal, either through breeding or at birth, whereas individuals were considered low-risk that had no known exposure to a *T. asinigenitalis* positive animal but which were either co-owned or managed by a person with a *T. asinigenitalis* positive premises.

All blood samples and culture swabs were collected by either an accredited veterinarian or by a federal veterinarian. Animals determined culture positive for *T. asinigenitalis* remained under quarantine and were required to undergo treatment as prescribed in VS Memoranda 558.3 and 558.4 and described by Kristula and Smith (2004). Carrier stallions and jacks were required to be test bred after completion of the cleaning regimen and their test mares to remain culturally and serologically negative for *T. equigenitalis* prior to being released from quarantine. In light of the identification of positive animals which were epidemiologically linked, the classification status for each animal was reevaluated periodically to ensure that adequate testing for *T. equigenitalis* was being performed.

Due to the concern that mares could harbor *T. asinigenitalis* during pregnancy, pregnant mares remained under quarantine until shortly after foaling. Within 7 days of foaling, swabs were collected from the clitoral fossa, clitoral sinuses and endometrium of the mares and a single swab collected from either the prepuce of male foals or from the clitoral region in female foals and tested to confirm their culture-negative status.

Because nurse mares represent a specialized segment of the horse industry that would normally not be included in routine surveillance testing, the Kentucky Department of Agriculture recommended to farm managers and private practitioners that all teaser stallions and nurse mares residing on commercial breeding operations be tested prior to the 1998 breeding/foaling season.

2.2. Diagnostic procedures

All nurse mares were visually inspected for evidence of current or recent vaginal discharge at the time of specimen collection. At the same time, the external cervical os was examined for evidence of inflammatory changes using a sterile single-use speculum. Specimens for bacteriological examination were collected using sterile swabs and each swab placed in an individual tube containing Amies transport medium with charcoal. All swabs were maintained on wet-ice or frozen freezer packs and delivered to the laboratory within 6 h of collection. Swabs were cultured onto non-selective and selective media (Timoney et al., 1982) which were incubated in 7% CO₂ for 7–10 days at 37 °C. Colonies with morphologic and growth characteristics typical or highly suggestive of *Taylorella* sp. were further evaluated by checking for catalase and cytochrome oxidase activity and by slide agglutination testing. All isolates were determined to be streptomycin sensitive by procedures described elsewhere (Fales et al., 1979). Isolates with growth and biochemical characteristics of *T. equigenitalis* were sent to the National Veterinary Services Laboratories, Ames, IA for confirmation of identity. At a later date, sequence analysis of the DNA encoding the 16S ribosomal RNA confirmed that the isolates were closely related but not identical to *T. equigenitalis* (Jang et al., 2001).

Blood samples were collected via jugular venipuncture, the sera harvested and checked for CF antibodies to *T. equigenitalis*.

2.3. Data analysis

Premises name, animal name and markings, risk classification, specific sites cultured, sampling dates, culture/serological results, and dates of quarantine release were entered into Microsoft Access 2000 (Microsoft Corporation, Redmond, WA, USA). The information was used to manage movement restrictions, trace contacts, track test results and conduct follow-up assessments.

Descriptive statistics and univariate analysis were conducted using SAS version 9.0 (SAS Institute, Cary, NC, USA) and Epi Info version 5.0 (CDC, Atlanta, GA, USA). Odds ratios (ORs) and 95% exact confidence intervals (CIs) were calculated.

3. Results

Between January and June 1998, a total of 58 individual premises were linked to the outbreak investigation through laboratory testing or contact tracing. Of these, 44 were commercial purebred breeding operations which were currently leasing or had leased nurse mares or teaser stallions from the owner of the culture-positive grey jack within the past year. Of the other 14 premises, 12 (86%) were under direct ownership or control of the owner of the grey jack. The two remaining premises under investigation consisted of the farm where the culture-positive grey jack was initially identified and a second nurse mare provider that had utilized the same grey jack to breed four of his 10 mares. Based on test results and breeding records, the epidemiological investigation focused primarily on the 14

premises with an established exposure risk to a *T. asinigenitalis* culture-positive animal. The diagnostic evaluation of the 44 commercial premises was carried out in accordance with testing recommendations for screening teaser stallions and nurse mares as prescribed by the Kentucky State Veterinarian.

A total of 217 equids were resident on the 14 premises on which animals were determined to have an increased exposure risk of acquiring *T. asinigenitalis*. Of these, the owner of the culture-positive grey jack managed a herd of 186 breeding adults on his 12 premises. The breed composition represented in these 12 herds was draft or draft crosses and included 172 (92.4%) females and 14 (7.5%) intact males of which only two were donkey jacks. Herd records indicated that 59 nurse mares were leased to 39 separate farms the previous foaling season, all of which were screened for CEM. None were culture positive for *T. asinigenitalis*. Additionally, 10 nurse mares and five teaser stallions belonging to the owner of the grey jack were located off-site under lease to nine different farms. All were traced and confirmed negative for *T. asinigenitalis* infection.

Using the risk criteria established for regulatory monitoring, 19.8% (43/217) of the animals were classified as high-risk and 80.2% (174/217) as low-risk based on exposure to a known *T. asinigenitalis* positive animal. Ten of the 43 high-risk animals (23.2%) were culture positive for *T. asinigenitalis* on at least one culture swab obtained during the course of the investigation (Fig. 1). Breeding records indicated that, for the 43 high-risk animals, 18 and 20 mares were bred by a stallion and a donkey jack, respectively. Overall, 18.4% (7/38) of mares bred to a carrier stallion or donkey jack became culture positive.

Excluding the 59 mares that were bred off-site, a total of 142 mares were bred by either a stallion or donkey jack owned by the nurse mare provider. Of the 20 mares bred to a donkey jack, five (25%) were culture positive. In contrast, only two of the 122 (1.6%) mares bred to stallions were culture positive. Overall, the risk of a mare being culture positive for *T. asinigenitalis* was 20 times greater for a mare bred to a donkey jack (estimated OR=20.0; exact 95% CI=2.9, 218.3) compared to a mare that was bred to a stallion.

The 10 animals that were culture positive for *T. asinigenitalis* resided on five of the 14 premises under intensive surveillance. These 10 animals consisted of three breeding males: two donkey jacks and one Paint Quarter-horse stallion, and seven draft-type breeding mares. Of the seven culture-positive mares, five were bred to the grey jack, one was bred to the Paint and one resided in a pasture with two Quarter-horse stallions. Neither of the quarter-horse stallions sharing the same pasture with this *T. asinigenitalis* positive nurse mare were positive for *T. asinigenitalis* based on swabbing and test breeding. Of the nine non-infected premises, three utilized a donkey in their breeding programs. On the remaining six premises, all males were stallions of mixed breeding and pasture mating was the sole method used to breed mares.

None of the nurse mares associated with this occurrence of *T. asinigenitalis* infection reportedly exhibited any evidence of clinical disease, including those confirmed culture positive. A total of 162 swabs were collected from the seven

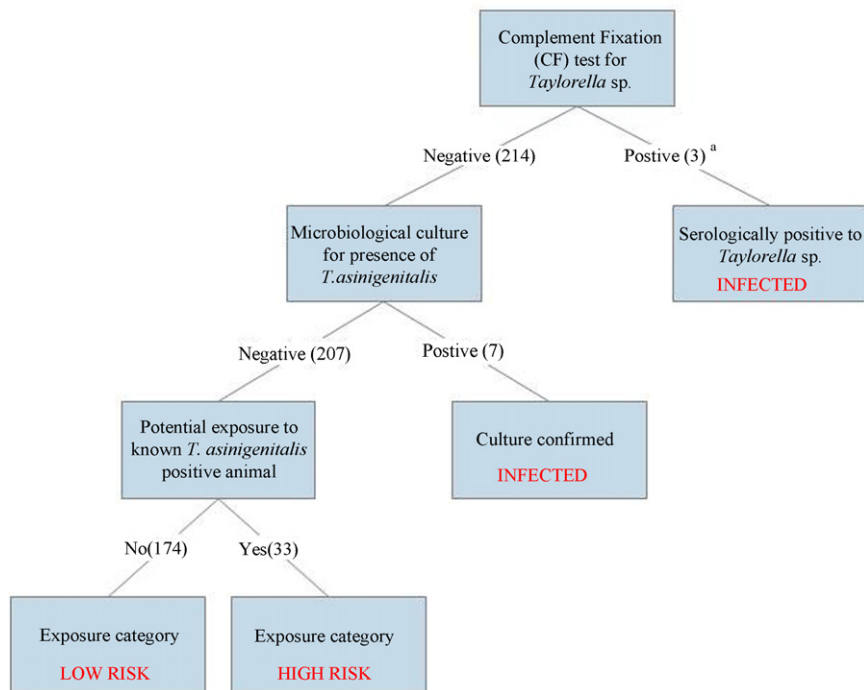


Fig. 1. Initial classification scheme for equids at risk for exposure to *T. asinigenitalis*. ^aThe three serological positive mares (CF titer $\geq 1:4$) were subsequently determined to also be culture positive.

infected nurse mares during the course of the investigation. Approximately 41% (20/49) of the swabs collected from the endometrium or external cervical os yielded isolates of *T. asinigenitalis* compared to 20.3% (12/59) and 18.5% (10/54) of specimens collected from the clitoral fossa and clitoral sinuses, respectively. Of the seven infected nurse mares, five (71.4%) remained culture positive following completion of 5 days of treatment with 4% chlorhexidine gluconate as a washing agent and 0.2% nitrofurazone as a topical ointment applied to the surface of the external genitalia. Of these five, two (40%) failed to clear the infection after a second round of treatment and were donated to the University of Kentucky for additional culturing and study; one remained culture positive in excess of 300 days. Following one course of treatment, all five breeding males previously culture positive for *T. asinigenitalis* were confirmed negative for the organism.

Voluntary compliance with recommendations proposed by the Kentucky State Veterinarian yielded an additional 616 sets – two to three swabs per set – of cultures and 500 blood samples for CF testing from a total of 592 randomly sourced nurse mares and teaser stallions leased to Thoroughbred and Standardbred farms. During the course of the investigation, only one additional culture-positive nurse mare was identified from this surveillance. This mare, which had been leased from the owner of the grey jack, was found to be culture positive upon arrival at the farm of destination. She had previously been classified as low-risk and subjected to culture and CF testing when the grey jack was first identified. Even after treatment in accordance with federal guidelines, the mare was still culture positive when sampled just prior to release from quarantine. Due

to time constraints on the resident veterinarian associated with the foaling season, the nurse mare lessee elected to have the mare clitoral sinusectomized prior to subjecting her to any additional treatments. Despite this, the mare was still culture positive when sampled after surgery. As a consequence, the entire group of horses that had commingled with this mare were reclassified as high-risk and subjected to additional regulatory testing, including test breeding of all stallions, before they were considered free of *T. asinigenitalis*. The additional testing did not identify any other infected animals.

In accordance with OIE published standards (OIE, 1998) the last infected premises were declared free of *T. asinigenitalis* on December 28, 1998. The entire investigation lasted over 300 days, being concluded after confirmation that two previously infected mares and their newborn foals were culturally and serologically negative for evidence of *T. asinigenitalis* infection.

4. Discussion

Identification of *T. asinigenitalis* positive animals was limited to those premises in Kentucky that were involved in acquiring and distributing nurse mares to commercial horse breeding operations. No Standardbred or Thoroughbred stallions or mares were found to be infected nor implicated in the spread of this organism, despite the fact that 74 nurse mares and teaser stallions were leased to such breeding farms by the owner of a *T. asinigenitalis* infected animal. The lack of demonstrable spread between these separate and distinct populations is not surprising as commercial breeding is highly controlled and there

is no economic incentive to breed animals of dissimilar type. Moreover, industry standards for reproductive management of commercial operations mandate the physical separation of mares and stallions. Because donkey jacks have to be hand mated to mares (Malcher, 2008), indiscriminate mating is unlikely to take place between these equid species. These observations suggest that, even though higher risk animals were present on multiple commercial breeding farms, the risk of transmission of *T. asinigenitalis* on these farms was extremely low. Overall, there would appear to be little opportunity for *T. asinigenitalis* to gain access to commercial breeding populations of Thoroughbreds or other horse breeds under appropriate conditions of management.

The availability of breeding and leasing records provided the opportunity to conduct extensive tracing to verify that all animals considered at risk were, in fact, traced and evaluated diagnostically and any culture-positive animals identified. It also provided a basis for a comparison of the transmission rates among carrier stallions across different outbreaks. While the observed rate of 18.4% is not significantly greater than the rate of 13.1% reported by Bryans and Hendricks (1979) for the 1978 outbreak of *T. equigenitalis* in Thoroughbreds, the rates may reflect differences in stallion management. It is customary for Thoroughbred stallions to be washed with a mild soap and water solution prior to breeding, a technique not used by the nurse mare provider. This observation might suggest that the use of this relatively simple management practice could contribute to a 28.8% (13.1 vs. 18.4) reduction in the transfer of this bacterium at time of breeding.

Consistent with the results of experimental studies with *T. equigenitalis* (Acland et al., 1983) the recovery rate for *T. asinigenitalis* was higher from swabs collected from the proximal than the distal reproductive tract. The observation that a majority of infected mares required more than one course of treatment is a cause for concern, suggesting that this organism may be more resistant to accepted forms of treatment in the breeding mare.

In contrast to the experimental findings of Katz et al. (2000), clinical signs consistent with an intra-uterine infection were not observed nor reported among any of the mares naturally exposed to *T. asinigenitalis* during this outbreak. These findings suggest that either the strain of *T. asinigenitalis* involved was only mildly pathogenic for horses or, if inflammatory changes did occur, they were mild and transient and were no longer evident at the time of specimen collection.

The source of the infection for the nurse mare identified through the voluntary testing of nurse mares and teasers remained unresolved. Neither the two stallions nor the other mares residing on the same premises with this mare were confirmed infected. It is likely that she was bred to one of the carrier donkey jacks prior to being leased, with the anticipation that she would not undergo further testing.

There is increasing evidence that *T. asinigenitalis* may be endemic in non-horse equids (personal

communications—P. Timoney) and risk factors for transmission of *T. asinigenitalis* are likely similar to those for *T. equigenitalis*. Animals that are maintained in direct contact with a *T. asinigenitalis* culture-positive donkey jenny or donkey jack would be at greater risk of acquiring infection provided they are treated similarly with respect to their reproductive management. Furthermore, the potential exists for indirect transmission of *T. asinigenitalis* through the use of contaminated instruments, etc., as exemplified during the first occurrence of CEM in the US in 1978 (Bryans and Hendricks, 1979). In the case of the culture-positive quarter-horse stallion it is possible that *T. asinigenitalis* was transferred from a donkey jack to the stallion through a breeding intermediate or through the use of shared equipment. This is similar to what has been speculated in the report by Båverud et al. (2006) and supports the belief that stallions are solely at risk for acquiring *T. asinigenitalis* if commingled on a property or utilized in a breeding program with a donkey jack that is a carrier of the organism.

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